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(57) Abstract

The present invention provides for the novel formulations of Camptothecin and its structurally related analogs in multilamellar or unilamellar vesicles. These novel formulations provide improved pharmacokinetics and pharmacodynamics for the compounds herein and thereby lowering the dose-dependent toxicity for use in anticancer treatments.

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CAMPTOTHECIN FORMULATIONS

FIELD OF THE INVENTION

This invention relates to liposomal formulations of Camptothecin and related analogs exhibiting reduced toxicity and improved efficacy.

BACKGROUND OF INVENTION

Several reports have appeared in the literature which describe anticancer drugs in liposomes not only for experimental cancer chemotherapy but also in clinical trials, the rationale being that liposomal encapsulation alters the pharmacokinetic parameters of the entrapped antineoplastics (see review by Daoud, S.S. et al; Liposomes in Cancer Therapy. Adv. Drug Deliv. Rev. 3: 405-418, 1989). Drug delivery advantages offered by liposomes include protection of liposome contents against the host, prolonged circulation/sustained release, and the possibility of targetting (passive, compartmental, ligand-mediated and physical).

To date, nearly all classes of anticancer drugs have been encapsulated in liposomes. These include: alkylating agents, nitrosoureas, cisplatin, antimetabolites (cytosine arabinoside, methotrexate, 5-fluoro-uracil) and anthracyclines. Studies with liposomes containing anthracycline antibiotics have clearly shown reduction of cardiotoxicity and dermal toxicity and prolonged survival of tumor-bearing animals compared to controls receiving free drug (Sells, R.A. et al. Cancer Treat. Rep. 14: 383-387 (1987)). Doxorubicin, a widely used anthracycline antibiotic is now in Phase III clinical trials.

The anticancer drug camptothecin and a related analogue, topotecan, a water-soluble analogue, are among a promising group of antineoplastics exhibiting a broad spectrum of activity against several human tumors. This class of therapeutic agents are specific inhibitors of topoisomerase I, an enzyme that intimately involves in DNA replication as it relieves the torsional strain introduced ahead of the moving replication fork. Inhibition of this enzyme results in lethal DNA damage during the course of replication. Inhibitors of topoisomerases are unique in that they do not cause cytotoxicity by depleting the product of their target enzymes, but by producing DNA damage by interfering with topoisomerase function (Hertzberg, R.P et al; Biochemistry 28: 4629-4638, (1989)).

Camptothecin, the parent compound, is both insoluble and unstable in water, undergoing a rapid pH dependent but reversible hydrolysis to the inactive carboxylate ion that is rapidly cleared in vivo. The lactone to open ring conversion is particularly favored at

alkaline pH and the open form predominates at physiological pH (7.4). Topotecan, a water-soluble analog of camptothecin, now in Phase II clinical studies, is also inactivated through reversible lactone hydrolysis and conversion to the carboxylate form (Underberg, W. J. M. et al; J. Pharmac. Biomed. Analysis 8: 681- 683, (1990)).

As with all antineoplastic drugs, the clinical use of camptothecin and topotecan is limited by a dose-dependent toxicity. In mice, the determined toxic dose for camptothecin in a suspension is 12 mg/kg and for a saline solution of topotecan (pH 3.0) is 15 mg/kg, respectively. Based on reported work with other liposomal drugs, it is anticipated that the pharmacokinetics and pharmacodynamics of camptothecin/topotecan would change in a liposome formulation. Thus, improving both the stability and antitumor activity of camptothecin and related analogues formulated in liposomes would certainly increase the commercial viability of these anticancer agents.

SUMMARY OF THE INVENTION

The present invention provides for the novel liposomal formulations of camptothecin and its structurally related analogues. These novel formulations provide improved pharmacokinetics and pharmacodynamics for the compounds herein and thereby lowering the dose-dependent toxicity for use in anticancer treatments.

More specifically, the formulations of camptothecin and its structurally related analogues are in the form of multilamellar vesicles dispersed in an aqueous phase with at least 80% of the total drug present incorporated in the lipid bilayer of the liposome at a high drug to lipid ratio (molar) of at least 1/100, preferably of about 1/10, and the lipid is of natural sources.

DETAILED DESCRIPTION OF THE INVENTION

It is well known to those skilled in the art that bioactive molecules can be encapsulated into liposomes, a man-made lipid/aqueous spherical structure. A liposome is defined as a structure consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartments. The number of bilayers is dependent on both the lipid composition and method of preparation. With additional energy, multilamellar vesicles (MLVs) can form unilamellar vesicles (ULVs). Many patents and scientific papers on liposomes have been published and the technical aspects of liposome formation and drug encapsulation are well known in the art (for review see Cullis P. R. et al; Generating and loading of liposomal systems for drug-delivery applications. Adv. Drug Deliv. Rev. 3: 267-282, 1989).

The present invention provides for novel liposomal formulations of camptothecin, and its structurally related analogues, to be administered parenterally, in the form of multilamellar vesicles or unilamellar vesicles dispersed in an aqueous phase, preferably

with at least 80% of the drug incorporated in the lipid bi-layer if it is a lipid-soluble drug, at a drug to lipid ratio (molar) of at least 1/100, preferably from 1/50 to 1/5 and more preferably from 1/20 to 1/10. Preferably the total lipid concentration is from 10-1000 mg/ml, preferably from 100-500 mg/ml, more preferably from 200-400 mg/ml, and still more preferably from 100-200 mg/ml. Preferably the vesicle is multilamellar. The particular size can be altered and readily determined by one skilled in the art. The particle size for such administration is generally from 0.5 microns to 10 and preferably from 0.2 - 2.0 microns. For intravenous administration the particle size is preferably less than 0.2 microns.

The multilamellar vesicles of the present invention can be prepared by standard methods (Cullis, P. R. et al; Generating and loading of liposomal systems for drug-delivery applications. Adv. Drug Deliv. Rev. 3: 267-282, 1989) whose disclosure is incorporated by reference in its entirety herein. One particular method that is suitable for lipophilic drugs involves: a) dissolving the lipids and drug in organic solvent(s) in a suitable lipid flask, b) slowly removing the organic solvents under vacuum to deposit on the inside walls of the lipid flask a dried lipid-drug film, and c) hydrating the dried lipid-drug film with an aqueous buffer or saline followed by mechanical agitation, such as vortexing, to disperse the drug-lipid suspension and produce the liposomes. These multilamellar liposomes can be freezed-thawed and then sized down, if necessary, by extrusion through polycarbonate membranes (Cullis P. R. et al; Adv. Drug Deliv. Rev. 3: 267-282, 1989) the disclosure of which is incorporated herein by reference in its entirety. In another preferred method, the lipophilic drug can be mixed with lipids using an ionizable hydrating agent to form a "preliposome gel". Following dilution of the gel with an aqueous solution, liposomes can be formed incorporating the lipophilic drug (EPO application No. 86306014.1, published 25 Feb. 1987, now US Patent No. 5,230,899) the disclosure of which are incorporated herein by reference in their entirety.

Preferably, the lipid may be obtained as a crude extract from natural sources. The crude extract comprises many different components, such as those indicated in the two tables below. (Paltauf et al., "Phospholipids- Natural, Semisynthetic, Synthetic" in Phospholipids:Biochemical, Pharmaceutical and Analytical Considerations, pp 1-12, Plenum Publishers, NY (1990)).

Table A

Phospholid Composition (%) of Soybean and Egg Yolk Lecithin

Phospholipid Class	Soybean	Egg Yolk
Phosphatidylcholine	31.7	78.8
Phosphatidylethanolamine	20.8	17.1
Phosphatidylserine	3.0	trace
Phosphatidylinositol	17.5	0.6
Phosphatidic acid	2	trace
Plasmalogen	-	1
Sphingomyelin	-	2.5
Other phospholipids	10.2	-
Phytoglycolipids	14.8	-

Table B

Fatty Acid Compostion (%) of Phosphatidylcholine From Soybean and Egg Yolk

Fatty Acid	Soybean	Egg Yolk
C16:0	14	31
C16:1	-	1
C18:0	4	13
C18:1	10	30
C18:2	64	15
C18:3	7	0.5
C20:4	-	4
C22:6	-	3

For purposes herein the crude extract is often highly purified to a single component if desired, i.e. such as lecithin. Enrichment of the extract with a particular lipid, such as phosphatidylglycerol, via a transphosphatidylation reaction catalyzed by the enzyme phospholipase D, while best be performed on a highly purifed extract, it may also be used on the crude product, resulting in egg phosphatidyl glycerol (EPG), for instance, in an egg yolk lecithin which is commercially available.

If the lipid contains phosphorus, i.e. is a phospholipid, then it is preferably fluid at ambient tempartures, more preferably at physiological temperatures.

Optionally, the mixture may contain a small blend of synthetic lipids. Synthetic, for use herein, means a homogeneous lipid which is not found in natural sources, such as the di-saturated phospholipids dimyristoyl-, dipalmitoyl-, and distearoyl-.

The lipids for use herein may contain unsaturation. This unsaturation may be on a single fatty acyl chain or in both chains (where applicable), the chain may include more than one double bond (i.e., di-unsaturated) or the chains may be partially hydrogenated, or contain mixtures thereof. Preferably if the phospholipids are unsaturated, then they are di-unsaturated products, such as di-oleoyl (C18:1) or di-linoleoyl (C18:2) phosphotidylcholine or glycerol. Preferably, the lipid bilayer contains lipids which may have at least 30% (molar) unsaturated fatty acyl chains. The term "fatty acyl chain" as used herein is the ester of a fatty acid. Alternatively, the term "fatty acid chain" may be used.

The lipids used herein may be of negative, neutral, or positive charge. Such products, include, but are not limited to, lipids such, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, disphosphatidylglycerol (cardiolipin), phosphatidic acid, phosphatidylinositol, sphingolipids, glycolipids, sulfatides, lysolipids and fatty acids, sterols such as cholesterol, polymerizable lipids, and combinations thereof.

Commercial products, such as, soy and egg sources are preferred, preferably soy and egg lecithin. Phospholipids, such as phosphatidylglycerol which can be obtained from egg lecithin are also commercially available. Suitable hydrogenated lecithins are products under the trade name Phospholipon H and G, (American Lecithin, Danbury CT); as well as Asahi's Type 5, 20, 40 and 65 egg phosphatides (Asahi Chemical Industry Co., Tokyo, Japan). Other lecithin products for use herein are Centrophase 31 and Centrolex P (Central Soya, Fort Wayne, IN).

At physiological conditions, lecithins and phosphatidylglycerols are bilayer-forming lipids, whereas certain types of phospholipids, such as phosphatidylethanolamine and cardiolipin, sphingolipids, sulfatides, lysolipids, glycolipids and glycerides, and fatty acids do not from stable bilayers. Therefore, the ratio of the bilayer-forming lipids to the non-bilayer-forming lipids can vary from 9:1 to 1:1, preferably from 9:1 to 7:1.

For use herein phosphatidylcholine and phosphatidylglycerol derivatives include, but are not limited to natural soybean or egg-yolk; or hydrogenated products obtained by hydrogenation of the natural soybean or egg-yolk phosphatidylcholine and phosphatidyl-glycerol. Suitable phosphatidylglycerol derivatives are egg phosphatidylglycerol or salts forms thereof.

Semi-synthetic products, such as dimyristoyl-, dipalmitoyl-, distearoyl-, dioleoyl-, dilinoleoyl, and mixtures thereof, such as 1-palmitoyl-2-oleoyl-, for instance, may be

added. Preferably if a semi-synthetic product is used it is 1-palmitoyl-2-oleoyl-, dioleoyl- or di-linoleoyl; or mixtures thereof. Preferably, the polar head group for the lipids is choline or glycerol. If a single component or an admixture of these two are used, it may be in amounts similar to that of their natural analogs. Preferred components are 1-palmitoyl-2-oleoyl- phosphatidylcholine or phosphatidylglycerol, dioleoyl phosphatidylcholine or phosphatidylglycerol (DOPC or DOPG). Preferably, if desired, the other di-saturated semi-synthetic components may be added in small amounts. Small amounts for use herein is less than 10% (molar), preferably less than 5% (molar).

Small amounts of other synthetic disaturated lipids, such as phosphatidylcholine and phosphatidylglycerol derivatives with C-14, C-16 and C-18 fatty acyl chains; or lipids with ester-linked fatty acids such as the mono-, di-, and tri-glycerides, or combinations thereof, may also be added.

Preferred lipids are the phosphatidylcholines (PC's) and phosphatidylglycerols (PG's) having

a) mono-unsaturated (C18:1) or (C18:2) fatty acyl chains;

b) di-unsaturated with (C18:1) or (C18:2) fatty acyl chains;

c) one particular class of mono-unsaturated PC or PG's are those having long (C16 or greater) saturated fatty acyl chain, on the 1-position of the glycerol backbone, with oleic or linoleic acid being esterified on the 2-position. One such product is 1-palmitoyl-2-oleoyl. This class is particularly preferred, since the fatty acid composition in these phospholipids is similar to the fatty acid distribution found in phospholipids from natural sources.

Preferred disaturated phospholipids are dimyristoylphosphatidylglycerol (DMPG), and dimyristoylphosphatidylcholine (DMPC).

The lipid bilayer may preferably include lipids selected from phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, cardiolipin, phosphatidic acid, and sterols such as cholesterol or combinations thereof. More preferably the lipid bilayer includes phosphatidylcholine, phosphatidylglycerol, and sterols such as cholesterol or combinations thereof.

Suitable neutral lipids include, but are not limited to, phosphatidylcholine and sterols, such as cholesterol and cholesterol derivatives thereof.

Suitable negatively charged phospholipids include, but are not limited to such as phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, phosphatidic acid and disphosphatidylglycerol (cardiolipin) and cholesterol analogs, such as cholesterol sulfate and hemisuccinate.

Suitable positive lipids include, but are not limited to stearylamine, or sphingosine.

The lipids preferably are not solely a sterol derivative but will include a sterol derivative, such as but not limited to, cholesterol and cholesterol analogs. The lipid formulation may additionally comprise a negatively charged cholesterol salt such as cholesterol sulfate or hemisuccinate which may be in combination with a neutral sterol as well.

The formulation may also contain additional ingredients such as antioxidants, i.e. vitamin A (α-tocopherol) or tocopherol-hemisuccinate, or other conventional antioxidants may be used.

The term "polymerizable lipids" is well known to those of skill in the art and suitably these lipids are used to help keep the aggregation and fusion down to a minimum for the sonicated vesicles by covalently linking the individual lipid molecules which form the membrane of the liposome. Suitable groups for such polymerization include, but are not limited to, vinyl, acryloylic, methacryloylic, butadienic, diacetylenic and $H_2NCC(O)OCH_3$. The reactivity of the polymerizable group is influences by the state of the bilayer. Preferably, the linking group is a diacetylenic moiety. Phospholipids containing diacetylene groups may do so in one or both acyl chains, for instance. Phosphatidylcholines containing a diacetylene group polymerize when dispersed as large multi- or unilamellar vesicles, but not as small ones. These polymerizable groups may be placed in all parts of the surfactant molecule, for the polar head to the extremity of the hydrocarbon chain. Cross-linking of the constituent molecules of a vesicle has been shown to increase the stability of the vesicle. A general review of polymerizable lipids may be found in Liposome Technology, Vol. 1, Preparation of Liposome, Chapter 9, Gregoriadis, Gregory, (1984) CRC Press whose disclosure is herein incorporated by reference.

The surface of the liposomes may also be modified with a polymer, such as, for example with polyethylene glycol (PEG), using procedures readily apparent to those skilled in the art. (Woodle, M.C. and Lasic, D.O., Biochim. Biophys. Acta **1113**:171-193, 1992; or Blume et al., Biochim. Biophysica Acta, **1029**: 91-97, 1990). Modifications of the liposome may be made by using any type of lipid available which does not have deleterious effects to the mammal, provided the lipid or combination of lipids along with other materials incorporated within the lipid matrix, should form a bilayer phase under physiologically relevant conditions. For a person skilled in the art it should be recognized that the composition of the liposomes may be modified to modulate the biodistribution and pharmacokinetics of the resulting liposomes.

Negatively charged liposomes may comprise from about 0% to about 50% (molar) negatively charged lipid; and more preferably from about 0 to about 30% (molar) negatively charged lipid in the composition. The ratio of the neutral to negatively charged lipid can very from 9:1 to 1:1 preferably from 3:1 to 1:1, more preferably from 9:1 to 3:1. When

cholesterol is also incorporated into the liposome its levels can vary 0 to about 50%, preferably from about 10% to about 30%, and more preferably from about 15 to 25%. The ratio of neutral lipid to cholesterol can vary from about 8:1 to about 1:1, more preferably from 4:1 to 1:1.

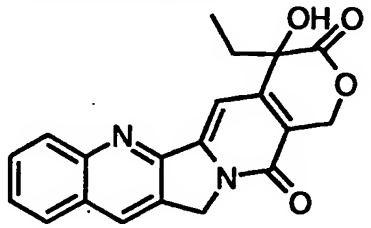
Preferred molar ratios of neutral:negative:sterol containing lipids include 4:2:4, 6:1:3, 4:3:3, and 5:4:1, respectively. Preferably, the neutral lipid is phosphatidylcholine, the negative lipid is phosphatidylglycerol and the sterol is cholesterol or a derivative thereof.

A preferred embodiment of the present invention is a pharmaceutical composition comprising Camptothecin or structurally related analogues thereof, in the form of multilamellar or unilamellar vesicles dispersed in an aqueous phase with a drug to lipid ratio (molar) of at least 1/100 and wherein the lipid is selected from phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, disphosphatidylglycerol (cardiolipin), phosphatidic acid, phosphatidylinositol, sphingolipids, glycolipids, sulfatides, lysolipids and fatty acids, sterols, polymerizable lipids, or combinations thereof;and wherein the lipid bilayer must contain either at least one charged lipid component present in an amount of at least 5% (molar), or the salt form of a water-soluble analog in an amount of at least 5% (molar); or mixtures thereof. The charged component is preferably negatively charged. Further the amount present of the negatively charged component is at least 10%, preferably at least 20% and more preferably at least 30%. The preferred negative component is a phosphatidylglycerol, charged salt of cholesterol, such as cholesterol sulfate, or hemi-succinate, or phosphatidylserine, more preferably egg phosphatidylglycerol (EPG). This formulation while usable for lipid soluble analogs is preferred for use herein for water-soluble analogs, such as topotecan. Optimally the water-soluble analog is not present in high amounts in the aqueous phase but in the lipid bilayer, preferably at least 30%, more preferably 50%, most preferably 80%.

In an alternative embodiment, the vesicle may consist of nonionic surfactants. This class of vesicles is known as "niosomes". Handjani-Vila et al., Int. J. Cos. Sci., 1:303-314; Baillie et al., J. Pham. Pharmacol., 37:863-868; van Hal, et al., Eur. J. Pharm. Biopharm, 38:47s; and Hofland et al., J. Contr. Rel. 16: 155-168, whose disclosures are incorporated by reference herein in its entirety. Nisomes have been prepared from several classes of non-ionic surfactants, e.g. polyglycerol alkylethers, glucosyl dialkylethers, crown ethers, and polyoxyethylene alkyl ethers. One possible lipid combination is the use of cholesterol and a non-ionic surfactant. Often a charged surfactant is intercalated in the bilayers in order to introduce electrostatic repulsion between the vesicles, thus increasing their stability.

By the term "camptothecin and related analogs or congeners" as used herein, means camptothecin and compounds which have the same core ring system with various substitutions but preferably have such modifications or substitutions in rings A & B.

The basic structure for camptothecin is



ring (A) (B) (C) (D) (E)

Many modifications have been made but in preferred for use herein are the following analogues: 9-aminocamptothecin, 9-nitrocamptothecin, 9-hydroxy-camptothecin, 10-hydroxy camptothecin, 10-amino camptothecin, 9-hydroxy-10-dimethylaminomethyl camptothecin, 20-(RS)-10,11-methylendioxycamptothecin, 9-chloro-10,11-methylenedioxy-(20S)-camptothecin, and 7-ethyl-10-hydroxycamptothecin and its derivatives, such as 7-ethyl-10-[[[4-(1-piperidino)-1-piperidino]carbonyl]-oxy]camptothecin. Preferably, for use therein the analogue is a lipid soluble analogue.

Since the initial isolation of *Camptotheca acuminata* of the novel alkaloid camptothecin in 1966 many related compounds have been made and are well known to those skilled in the art. It would be difficult to list all of the papers and patents covering such compounds but a representative grouping of reference showing additional congeners useful herein include but are not limited to, Wall et al., J. Med. Chem., Vol. 29, pp 1553-1555 (1986) disclosing 11-hydroxycamptothecin; US Patent No.'s 4,981, 968, 4,894,456 and 5, 122,526 to Wall et al.; Hertzberg et al., J. Med. Chem., Vol. 32, pp 715-720 (1989) disclosing modifications of the hydroxy lactone ring of camptothecin; Wani et al., J. Med. Chem., Vol. 23, p 554 (1980), Wani et al., J. Med. Chem., Vol. 30, p 1774 and pp 2317-2319 (1987); Wani et al., J. Med. Chem., Vol. 29, p 2358-2363 and pp 1553-1555 (1986); JP 51-91297, CA 84: 115629; Derwent Abstract 85-034354/06 (Yakult Honsha KK) to Japanese Patent 59-227884, Derwent Abstract 87-281010/40 (Yakult Honsha KK) as JP 62-195384; EPO 0088542; US Patent Nos. 4,914,205, 4,473,692, 4,545,880, 4,064,462, and US Patent No. 5,004,758 ; whose disclosures are incorporated by reference herein in their entirety.

In the case of water-soluble analogs of Camptothecin, such as topotecan, a prior modification either of the lipid composition and/or charge of the liposome, or lipid derivatization of the analog may be necessary to improve incorporation into the liposomal lipid bilayer.

Modification of the lipid composition of the liposome means herein, increasing the amount of negatively charged lipid or lipids present in the liposome; modifying the degree of fatty acid chain unsaturation and altering the levels of cholesterol in the bilayer. Modification of the charge of the liposome means herein, increasing the levels of the negatively charged lipids such that the overall liposome charge is increased, i.e. on the surface.

A water-soluble analog of camptothecin, herein means an analog or drug which is charged when in the physiological pH range.

Modification of the water-soluble analog by lipid derivatization means herein, conjugating the drug to a lipid moiety, wherein the lipid has an opposite charge to that of the drug, thus forming an ion-pair.

For water-soluble drugs having a positive charge, such as topotecan, negatively charged lipids, such as cholesterol sulfate or hemisuccinate may be employed. Formation of an ion-pair with a water-soluble analog may be prepared *in-situ* during liposome formation or prior to incorporation into the liposome.

By the term "parenterally" as used herein is meant by intravenous, intramuscular, subcutaneous, or intraperitoneal administration. It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of the formulations herein will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests. Prior treatment has generally accorded that camptothecin be administered in a dosage range per course of therapy (one month) from 20 mg/M^2 to 800 mg/M^2. Prior treatment has generally accorded that topotecan, IV, be administered in a dosage range per course of therapy , daily X 5 days at 1.5 mg/M^2 for small cell lung carcinoma (SCLC) and Ovarian Cancer.

For instance, the drug concentration in the liposome, for camptothecin, may vary from 0.1 mg/ml to 10 mg/ml, preferably from about 4 mg/ml to 6 mg/ml. It is recognized in any instance, that the total volume of the liposome may be adjusted to meet the desired dose requirements. For example, for camptothecin administration of 60 mg/kg a liposome volume of 10 ml/kg was employed.

Recently, T.G.Burke et al; J. Am. Chem. Soc. **114**: 8318-8319, 1992; and Biochem., **32**: 5352-5364, (1993) have reported that small unilamellar vesicles of DMPC or DMPG can improve the stability of camptothecin and several analogues under physiological conditions by intercalation of the drug's lactone ring into the lipid bilayer, thus isolating it from solution (PBS, pH 7.4). While the authors have demonstrated

binding and stabilization of camptothecin at very high lipid to drug ratio (2.9×10^5), this is not commercially attractive where a high drug loading in the formulation is highly desirable. Liposomes, however, may provide the efficient delivery system needed without compromising the drugs antitumor activity.

EXAMPLE 1

Preparation of camptothecin-containing DMPC:DMPG multilamellar liposomes

1.440 g or 0.540 g of dimyristoylphosphatidylcholine (DMPC) were mixed with 0.160 g or 0.060 g dimyristoylphospatidylglycerol (DMPG) sodium salt and without or with 0.018 g camptothecin, respectively, in two separate lipid flasks. Six ml of methylene chloride:methanol (9/1, v/v) were added to solubilize camptothecin plus sufficient volume of chloroform (5-10 ml) to completely solubilize the lipids and produce a yellowish solution. The drug-free lipids were treated in a similar manner. The organic solvents were subsequently removed under vacuum in a rotary evaporator to leave behind a thin film of the drug-lipid mixture. Residual solvents were removed in dessicator under vacuum overnight.

Hydration of the dried drug-lipid film along with a drug-free lipid sample was carried out at 37°C, a temperature well above the phase transition temperature (T_m) of the lipids. This temperature where phospholipids undergo a characteristic gel to liquid crystalline transition temperature is 23°C for DMPC or DMPG alone and in binary mixtures. The samples were hydrated with physiological saline, 5 and 8 ml for the drug-containing and drug-free samples, respectively, and allowed to equilibrate at 37°C for 15-30 min. Multilamellar liposomes were produced by vortex-mixing the lipid or drug-lipid films via 3-5 of 1-min vortexing cycles at maximum speed using a laboratory vortex-mixer (Vortex-Genie 2 from Scientific Industries).

The composition of the resulting liposomes was DMPC:DMPG (90:10) and DMPC:DMPG:Camptothecin (84.6:9.4:6.0) for the drug-free and drug-containing liposomes, respectively. The newly formed liposomes were further equilibrated at 37°C for about an hour and then stored at ambient temperature. The total lipid and drug concentration were 200 and 6 milligrams/milliliters (hereinafter referred to as mg/ml), respectively. If desired, these multilamellar liposomes can be sized down by extrusion through 0.4 μm and 0.2 μm polycarbonate membranes.

EXAMPLE 2

Preparation of camptothecin-containing EPC:EPG multilamellar liposomes

Using procedures analogous to those of Example 1 except that all operations were carried out at ambient temperature, the following multilamellar liposomes consisting of egg

phosphatidylcholine (EPC) and egg phosphatidylglycerol (EPG) sodium salt were prepared:

a) EPC:EPG (90:10); and

b) EPC:EPG:camptothecin (84.6:9.4:6.0)

at total lipid and drug concentration of 200 and 6 mg/ml, respectively.

EXAMPLE 3

Preparation of camptothecin-containing EPC:EPG:CHOL multilamellar liposomes

1.333 g or 0.425 g of egg phosphatidylcholine (EPC) were mixed with 0.171 g or 0.064 g egg phosphatidylglycerol sodium salt (EPG) and 0.167 g or 0.063 g of cholesterol (CHOL) in two separate lipid flasks and if present with 0.018 g camptothecin. Subsequent procedures were carried out at ambient temperature as described in Examples 1 and 2. The resulting liposomes were:

a) EPC:EPG:CHOL (70:10:20); and

b) EPC:EPG:CHOL: camptothecin (65.8:9.4:18.8:6.0) at total lipid and drug concentration of 200 and 6 mg/ml, respectively.

EXAMPLE 4

Determination of camptothecin Incorporation in Multilamellar Liposomes.

The amount of camptothecin incorporated in liposomes was determined by a slight modification of the method described by Constantinides, P.P. et al; Chem. Phys. Lipids 51: 105-118, (1989). Briefly, the multilamellar liposomes prepared as described in Examples 1-3, were equilibrated for 3 hrs at ambient temperature (EPC:EPC, 90:10 and EPC:EPG:CHOL, 70:10:20 with and without camptothecin) or 37°C (DMPC:DMPG 90:10, with and without camptothecin). The drug-free and drug-lipid mixtures were then centrifuged at 50,000 rpm for 2 hrs at 25°C using a Ti 80 fixed angle rotor. Under these conditions, the lipids separated from the aqueous medium and the concentration of camptothecin in the supernatant was determined upon proper dilution by measuring the absorbence at 253 nm using a Beckman DU70 spectrophotometer.

The amount of the drug in liposomes was calculated as follows:

$$D_L\ (\%\ \text{of total}) = (Ct - Cs/Ct) \times 100$$

where, D_L is the drug in liposomes, Ct is the absorbance of camptothecin before centrifugation (total drug present) and Cs is the absorbance of the supernatant depleted of camptothecin by the presence of the lipid. Absorbances were corrected for the amount of the lipid remaining in the supernatant which was less than 10% of the total lipid. The results from these lipid/partitioning studies are summarized in Table I.

Table I: Determination of Camptothecin Incorporation in Multilamellar Liposomes

Liposome Composition	Drug Incorporation (% of total)
DMPC:DMPG:camptothecin (84.6:9.4:6.0)	91
EPC:EPG:camptothecin (84.6:9.4:6.0)	98
EPC:EPG:CHOL:camptothecin (65.8:9.4:18.8:6.0)	97

As Table I shows, with all three liposome compositions very high drug loading was obtained which is certainly desirable for a commercially viable formulation.

Example 5

Particle Size Determination of Camptothecin Liposomes

The particle size of camptothecin liposomes along with the corresponding drug-free liposomes prepared as described in Examples 1 to 3 was determined upon a 400-fold dilution with physiological saline using a laser light scattering. A Malvern Photon Correlation Spectrometer model 4700 equipped with an argon laser model 2000 from Spectra Physics was employed to monitor particle size of the various liposomal formulations. Light scattering was monitored at 90° angle and temperature of 25°C. Polystyrene (latex) beads were used as particle size standard. The light scattering data is summarized in Table II.

Table II: Particle Size of Camptothecin-free and Camptothecin-Incorporating Multilamellar Liposomes

Liposome Composition	Particle Size[a]	
	Z average (μm)	Polydispersity
DMPC:DMPG (90:10)	1.151	0.784
DMPC:DMPG:camptothecin (84.6:9.4:6.0)	0.652	0.622
EPC:EPG (90:10)	1.015	0.645
EPC:EPG:camptothecin (84.6:9.4:6.0)	0.761	0.633
EPC:EPG:CHOL (70:10:20)	1.100	0.450
EPC:EPG:CHOL:camptothecin (65.8:9.4:18.8:6.0)	0.698	0.615
Latex Beads, 0.74 micron standard	0.799	0.129

[a]: based on monomodal analysis.

As expected for multilamellar liposomes, the average particle size of these liposomes was about 1 micron (μm) (Table II) with the size distribution varied between 0.5 to 5.0 μm. The size heterogeneity of these liposomes is also evident from the high polydispersity value as compared to that obtained with the latex beads standard. The polydispersity index is a measure of particle homogeneity. It varies from 0 to 1; the closer to zero the value the more homogeneous (monodispersed) the particles.

EXAMPLES 6

Incorporation of Topotecan into Multilamellar Liposomes at Low Lipid Concentration

The following stock solutions of egg phosphatidylcholine (EPC), egg phosphatidylglycerol (EPG) and cholesterol (CHOL) in chloroform, cholesterol sulfate (CHOL-S) and Topotecan in methanol and the hydrophobic salt of Topotecan with cholesterol sulfate (CHOL-S-TOPOTECAN) in chloroform:methanol (1:1) were employed: EPC (24.4 millimolar ; EPG (13.0 millimolar) ; CHOL (25.8 millimolar); cholesterol sulfate (13.7 millimolar) ; Topotecan (10.9 millimolar) and cholesterol-sulfate-Topotecan salt (2.3 millimolar) . Using procedures analogous to those of Example 1-3, saline pH 3.1 as the hydration solution and total lipid concentration of 2.0 mg/ml (2.7 millimolar) the following multilamellar liposomes were prepared at ambient temperature:

a) EPC:EPG:CHOL:Topotecan (48:19:24:9);

b) EPC:EPG:CHOL:Topotecan (37:30:24:9);
c) EPC:EPG:CHOL:CHOL-S:Topotecan (48:19:15:9:9);
d) EPC:CHOL:CHOL-S: Topotecan (67:24:4.5:4.5);
e) EPC:CHOL:CHOL-S-Topotecan (67:24:9).

EXAMPLE 7

Incorporation of Topotecan into Multilamellar Liposomes at High Lipid Concentration.

Using the lipid and drug stock solutions of Example 6 and procedures analogous to those of Examples 1-4, multilamellar liposomes incorporating Topotecan (compositions a-e in Example 6) were also prepared at high lipid concentration (200 mg/ml or 270 millimolar). A drug to lipid ratio (molar) of 1 to 11 was maintained at both low and high total lipid concentration.

EXAMPLE 8

Determination of Topotecan Incorporation into Multilamellar Liposomes of Examples 6 & 7

The method outlined in Example 4 was employed to quantitate the level of Topotecan incorporated into multilamellar liposomes of Examples 6 and 7. The results from these studies are summarized on Table III:

Table III: Determination of Topotecan Incorporation in Multilamellar Liposomes at Low and High Lipid Concentration

Liposome Composition (mol %)	Drug Loading (% of total)	
	Low Lipid Conc. (2.0 mg/ml)	High Lipid Conc. (200 mg/ml)
EPC:EPG:CHOL:Topotecan (48:19:24:9)	48	80
EPC:EPG:CHOL:Topotecan (37:30:24:9)	75	79
EPC:EPG:CHOL:CHOL-S:Topotecan (48:19:15:9:9)	40	70
EPC:CHOL:CHOL-S:Topotecan (67:24:4.5:4.5)	30	ND
EPC:CHOL:CHOL-S-Topotecan[a] (67:24:9)	34	ND

a: A 1:1 stoichiometry of the salt has been confirmed by chemical analysis.

The data in Table III indicates that: a) increasing the level of the negatively charged component in the liposomes (EPG) significantly increased the degree of drug incorporation, b) at EPG levels lower than 30 mol%, the higher the lipid concentration the higher the drug incorporation, c) in the absence of EPG, significant drug incorporation was obtained with cholesterol sulfate as the negatively charged component in liposomes, the small differences between EPG- versus CHOL-S-containing liposomes being due to the different levels of the two lipids present, and d) essentially the same degree of drug incorporation was obtained using either the preformed salt of topotecan with cholesterol sulfate or by forming *in situ* the salt during liposome preparation.

EXAMPLE 9

The particle size and polydispersity of drug-free and topotecan-incorporating liposomes was determined by laser light scattering as described in Example 8. Table IV summarizes the light scattering data:

Table IV: Particle Size and Polydispersity of Drug-Free and Topotecan-Incorporating Multilamellar Liposomes[a]

Liposome Composition	Particle Size[b] Z average (microns)	Polydispersity
EPC:EPG:CHOL (48:19:24)	2.111 (1.343)	0.290 (0.447)
EPC:EPG:CHOL:Topotecan (48:19:24:9)	2.127 (0.982)	0.138 (0.666)
EPC:EPG:CHOL (37:30:24:9)	(2.266)	(0.741)
EPC:EPG:CHOL:Topotecan 37:30:24:9)	(1.703)	(0.675)
EPC:EPG:CHOL:CHOL-S (48:19:15:9)	3.330 (1.325)	0.331 (0.592)
EPC:EPG:CHOL:CHOL-S:Topotecan (48:19:15:9:9)	3.027 (0.738)	0. 331 (0.682)
Latex Beads, 0.74 microns standard	0.799	0.129

a: based on monomodal analysis.

b: numbers in parentheses are obtained at low lipid concentration (2.0 mg/ml). Other values were obtained at high lipid concentration (200 mg/ml). Free-topotecan was removed from all liposome samples before particle size determination.

At low lipid concentration, Topotecan reduced the particle size of the corresponding liposomes whereas, at high lipid concentration, small differences in particle size were observed between drug-free and drug-containing liposomes. Once again, the size heterogeneity of these liposomes is also evident from the high polydispersity value as compared to that obtained with the latex beads standard. Topotecan appears to have a small but variable effect on the polydispersity.

EXAMPLE 10

Toxicity Evaluation of Liposomal Camptothecin

Liposomally formulated camptothecin (Examples 1-3) was evaluated for toxicity in B6D2F1 mice upon intraperitoneal administration as a single bolus injection on days 1 and 5 and comparison to a suspension formulation of camptothecin (N,N-dimethyl-acetamide:Cremophor EL: Water, 10:10:80, v/v). Dosing volume and total lipid dose were maintained at 10 ml/kg and 2.0 g/kg, respectively. No apparent toxicity was observed when drug-free liposomes were dosed at 2 g/kg. In fact, animals gained some weight in response to lipid administration. Camptothecin in liposomes was dosed at 60, 30, 15, 7.5 and 3.75 mg/kg and in suspension at 24, 12, 6, 3 and 1.5 mg/kg. Toxicity evaluation was based on clinical signs i.e number of survivors and body weight lost during a three-week study. The results from these studies are summarized in Table V:

Table V: Comparison of the Toxicity of Camptothecin Formulated in Liposomes or Suspension upon i.p. Administration to Mice[a]

Formulation	MTD[b] (mg/kg)	Survivors	Change in BW[c] Day 4	Day 8
DMPC:DMPG:camptothecin (84.6:9.4:6.0)	30	5/5	-3.0	-3.8
EPC:EPG:camptothecin (84.6:9.4:6.0)	> 60	5/5	-0.8	+0.2
EPC:EPG:CHOL:camptothecin (65.8:9.4:18:6.0)	> 60	5/5	-1.4	-1.4
DMA:Cremophor EL:Water (10:10:80) with camptothecin at 2.4 mg/ml	6	5/5	-1.0	-1.0

a: Five animals per group
b: Maximum Tolerated Dose
c: Body Weight

EXAMPLE 11

Efficacy Evaluation of Liposomal Camptothecin

Murine L1210 lymphocytic leukemia, obtained originally from the Frederick Cancer Research Center of the National Cancer Institute (Frederick MD), was maintained by serial intraperitoneal (i.p.) transplantation in syngenic DBA/2 mice according to a standard screening protocol by Geran et al; Cancer Chemother. Rep. 3: 1-103, (1972).

For efficacy studies, L1210 was harvested, pooled, diluted and counted using a ZBI Coulter Counter. The cell suspension was adjusted to $5x10^6$ cells/ml. An inoculum of 0.2 ml of L1210 was given intravenously (i.v.) via lateral tail vein into 20-22 g B6D2F1 female mice using a 25-gauge needle. Tumor inoculum was tested for bacterial contamination by 24-hr incubation in thioglycollate broth. Animals were randomized into groups of 5 and housed in shoe box cages. Food and water were provided *ad libitum*. All experiments included 3 groups of animals as untreated controls. A titration of tumor cells (10^1-10^5) in untreated animals was included so that drug-induced cell kill (NCK) could be calculated. Administration of liposomal and suspended camptothecin (see Example 6) commenced at Day 2 after tumor implantation. Camptothecin was administered i.p. as described in Example 6, that is at 4-5 dose levels, each being 50% of the preceding higher dose.

The results from an efficacy study are shown in Figure 1. Camptothecin administered i.p. as a single bolus injection on Days 2 and 6, was highly effective against i.v-implanted L1210 lymphocytic leukemia. Two independent experiments were conducted. Untreated control mice survived for a median of 6 days. The maximum tolerated dose (MTD) for EPC:EPG liposomes with and without cholesterol was greater than 60 mg/kg. At 60 mg/kg, camptothecin in EPC:EPG liposomes increased lifespan by 233 and 200% in the two experiments which represents a reduction in tumor burden of > 6 log. In EPC:EPG:CHOL liposomes camptothecin prolonged lifespan by about 200 and 183% a decreased tumor burden averaging 5.8 log. The DMPC:DMPG formulated camptothecin had an MTD of 15-30 mg/kg. This formulation increased lifespan by 150 and 117% in the two experiments, an average tumor cell reduction of 3 log. Camptothecin formulated as an aqueous suspension had an MTD of 12 mg/kg which produced 133 and 117% ILS for an average net tumor cell kill of 2.7 log. Thus the EPC:EPG liposomal formulation reduced the toxicity of camptothecin without affecting antitumor activity. This resulted in greater efficacy at the higher dose levels achieved with the liposomal formulation.

EXAMPLE 12

Pharmacokinetic Study of Liposomal Camptothecin

EPC:EPG:camptothecin (84.6:9.4:6.0) liposomes at total lipid and drug concentration of 200 and 6 mg/kg, respectively, were administered i.p. as a single bolus dose of 60 mg/kg to B6D2F1 mice. Control mice received camptothecin in suspension (DMA: Cremophor EL: Water, 10:10:80) at either 10 or 60 mg/kg. Plasma samples were collected at 5, 15, 30, 60, 90, 120, 240, 360, 480, 600, 720, 840, 960, 1080, 1260 and 1440 min using a group of three animals per time point (with camptothecin in suspension at a dose of 60 mg/kg plasma samples were collected up to 240 min).

Plasma samples were assayed for total camptothecin (lactone and hydroxy acid) and lactone form by HPLC using a modification of the method by Grochow et al; Drug Metabolism Disposition 20; 706-713, 1992. Briefly, 100 ml of plasma was added to cold methanol, the samples were vortexed and then centrifuged at 14,000 rpm for 30 seconds. The supernatants were removed and assayed immediately or stored at -70°C. To quantitate total drug, 100 ml of the methanolic supernatant was added to 50 ml of 0.050 M phosphoric acid and incubated at room temperature for 2 hrs prior to chromatographic analysis. For lactone quantitation, 100 ml of the methanolic supernatant was added to 0.010 M sodium phosphate buffer (pH 6.0) and immediately injected into the HPLC.

Separation was accomplished using an isocratic HPLC system which consisted of a Series 4 liquid chromatograph (Perkin-Elmer) and a Beckman 157 fluorescence detector. One hundred ml injections were made using a WISP 710B autosampler (Waters) equipped with an ODS 4.6mm X 15cm 5mm Beckman column. Retention times and peak areas were recorded with an HP 3396 Series integrator (Hewlett Packard). The plasma drug concentrations were calculated using a calibration curve with concentrations ranging between 30 to 600 nM.

The results from the pharmacokinetic study are shown in Fig. 2. As can be seen high and sustained plasma levels of camptothecin were observed over a 4-hr period after administration of the liposomal formulation as compared to those obtained with the drug suspension. Significantly larger area under the plasma concentration-time curve (AUC) and longer elimination half-time ($t_{1/2}$) were obtained from liposomal versus suspended camptothecin. Despite this higher exposure there was reduced toxicity for the liposomal formulation. In figure 2 the CPT (EPC:EPG) 60mg/kg formulation had a terminal $t_{1/2}$ (min) of 369 and an AUC (μM/min) of 939; the CPT (Aqueous Suspension of 10mg/kg) had a terminal $t_{1/2}$ (min) of 840 and an AUC (μM) of 42.

Table VI - Effect of a Single IP Injection of Liposome Encapsulated Topotecan in Normal Mice

Topotecan Concentration mg/kg, Ip	Formulation	Δ Weight Day 3 gm	Δ Weight Day 4 (gm)	Δ Weight Day 5 (gm)	Day til Death	Survivors
200	A	-2.4	-3.4	-4.6	7-8	0/5
100	A	-2.2	-3.4	-4.2	7-9	0/5
50	A	-1.0	-2.2	-3.8		5/5
25	A	-1.2	-2.6	-3.2		5/5
12.5	A	-0.4	+0.0	+0.4		5/5
200	C	-1.6	-1.4	-	3-7	2/5
100	C	-2.4	-3.0	-4.4	8-10	0/5
50	C	-1.6	-2.8	-4.0	9-10	2/5
25	C	-1.0	-2.4	-2.8		5/5
12.5	C	-0.6	+0.2	+0.4		5/5
200	E	-3.0	-3.6	-	4-8	0/5
100	E	-1.8	-3.0	-4.0	8-9	0/5
50	E	-2.0	-3.2	-4.2	7-10	0/5
25	E	-1.6	-2.6	-3.4		5/5
12.5	E	-1.0	-1.6	-1.6		5/5
0	A	+0.8	+1.0	+1.2		5/5
0	C	+1.0	+1.0	+1.2		5/5
0	E	+0.2	+0.4	+0.6		5/5
69.5	ACID SALINE	-3.0	-4.0	-5.0	3-8	0/5
41.7		-2.6	-3.8	-5.0	7-9	0/5
25		-3.8	-4.4	-4.4		5/5
15		-1.8	-2.4	-2.0		5/5

Female BDF_1 mice weighed 22-26 gm at the start of the experiment
Formula A = EPC:EPG:CHOL (48:19:24) PLUS Topotecan (9)
Formula C = EPC:EPG:CHOL (36:30:24) PLUS Topotecan (9)
Formula E = EPC:EPG:CHOL:CHOL-S (48:19:24:9) PLUS Topotecan (9)

Table VII
Effect of Liposome Encapsulated Topotecan Administered IP on Days 2 and 6 in Mice Bearing Systemic L1210 Lymphocytic Leukemia

Topotecan Concentration mg/kg	Formulation	Δ Weight Day 6 (gm)	Δ Weight Day 9 (gm)	Median Survival Time (Days)	Increase in Lifespan (%)
60	A	-4.9	-	7	17 Tox
30	A	-4.6	-	8	33 Tox
15	A	-2.0	-2.2	9	50
7.5	A	-2.4	-3.6	20	233
3.75	A	+0.6	+0.8	16	167
60	C	-3.2	-	8	33 Tox
30	C	-3.6	-5.7	11	83 Tox
15	C	-3.1	-3.1	13	117
7.5	C	+0.4	0.0	17	183
3.75	C	+1.6	+1.3	13	117
0	A	-	-	6	0
0	C	-	-	6	0
30	ACID SALINE	-4.2	-6.4	10	67 Tox
15		-0.6	-0.2	19	217
7.5		+0.8	+0.6	14	133
3.75		+1.4	+1.2	11	83

Female BDF_1 mice weighed 18-21 gm at the start of the experiment
Formula A = EPC:EPG:CHOL (48:19:24) PLUS Topotecan (9)
Formula C = EPC:EPG:CHOL:CHOL-S (48:19:15:9) PLUS Topotecan (9)

List of Abbreviations

DMPC	Dimyristoylphosphatidylcholine
DMPG	Dimyristoylphosphatidylglycerol
EPC	Egg phosphatidylcholine
EPG	Egg phosphatidylglycerol
CHOL	cholesterol
CHOL-S	cholesterol sulfate
DMA	N,N-Dimethylacetamide
MLV	multilamellar vesicles
SUV	small unilamellar vesicles
MTD	maximum tolerated dose
ILS	increase in lifespan

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A pharmaceutical composition comprising Camptothecin or lipid-soluble structurally related analog thereof, in the form of multilamellar or unilamellar vesicles at a drug to lipid ratio (molar) of at least 1/100 and wherein the lipid is selected from phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, disphosphatidylglycerol (cardiolipin), phosphatidic acid, phosphatidylinositol, sphingolipids, glycolipids, sulfatides, lysolipids and fatty acids, sterols, polymerizable lipids, or combinations thereof.

2. The pharmaceutical composition, according to Claim 1, wherein the lipids may contain at least 30% (mol/mol) unsaturated fatty acyl chains.

3. The pharmaceutical composition, according to Claim 1, wherein the drug to lipid molar ratio is from 1/50 to 1/5.

4. The pharmaceutical composition, according to Claim 3, wherein the drug to lipid molar ratio is from 1/20 to 1/10.

5. The pharmaceutical composition, according to any of Claims 1 to 4, wherein the total lipid concentration is from 10 to 1000 mg/ml.

6. The pharmaceutical composition, according to Claim 5, wherein the total lipid concentration is from 100 to 500 mg/ml.

7. The pharmaceutical composition, according to Claim 1, wherein the lipid is a negatively charged lipid selected from phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, phosphatidic acid or diphosphatidylglycerol.

8. The pharmaceutical composition, according to Claim 1, wherein the lipid is a neutral lipid selected from phosphatidylcholines or sterols.

9. The pharmaceutical composition, according to Claim 1, wherein the lipid is a non-bilayer forming lipid selected from phosphatidylethanolamine, cardiolipin, sphingolipids, sulfatides, lysolipids and fatty acids, glycolipids or glycerides.

10. The pharmaceutical composition, according to Claim 1, wherein the lipid if non bilayer forming additionally comprises a lipid which forms bilayers in a ratio of 9:1 to 1:1 bilayer-forming lipids to the non-bilayer forming lipids.

11. The pharmaceutical composition, according to Claim 6, wherein the liposome may comprise from 0% to about 50% (mol/mol) negatively charged lipid.

12. The pharmaceutical composition according to Claim 1 wherein the ratio of the neutral to negatively charged lipid is from about 9:1 to 1:9.

13. The pharmaceutical composition according to Claim 12 wherein the ratio of the neutral to negatively charged lipid is from about 3:1 to 1:1.

14. The pharmaceutical composition according to Claim 12 wherein neutral to negatively charged lipid is from about 9:1 to 3:1.

15. The pharmaceutical composition according to Claim 2 wherein neutral lipid is phosphatidylcholine and the negatively charged lipid is phosphatidylglycerol.

16. The pharmaceutical composition, according to any of Claims 1 to 15, wherein the lipid further comprises a negatively charged sterol salt.

17. The pharmaceutical composition, according to Claim 1 or 15 wherein the lipid incorporates a sterol, the sterol being present in the liposome at levels of about 0 to about 50% (mol/mol).

18. The pharmaceutical composition according to Claim 17 wherein the lipid incorporates a sterol, the sterol being present in the liposome at levels of about 10 to about 30%.

19. The pharmaceutical composition according to Claim 18 wherein the lipid incorporates a sterol, the sterol being present in the liposome at levels of about 15 to about 25%.

20. The pharmaceutical composition, according to Claim 1, wherein the ratio of neutral lipid to sterol can vary from about 5:1 to about 1:1.

21. The pharmaceutical composition, according to Claim 1, wherein the surface of the liposome is modified by a polymer.

22. The pharmaceutical composition, according to Claim 21, wherein the polymer is polyethylene glycol.

23. The pharmaceutical composition, according to Claim 1, wherein the drug is Camptothecin.

24. The pharmaceutical composition, according to Claim 23, wherein the drug concentration varies from 0.1 to 10mg/ml.

25. The pharmaceutical composition, according to Claim 24, wherein the drug concentration varies from preferably 1 to 6 mg/ml.

26. The pharmaceutical composition according to any of Claims 1 to 25 wherein the lipid contains C18:1 or C18:2 phosphatidylcholine and/or phosphatidylglycerol, and optionally comprises cholesterol.

27. A pharmaceutical composition comprising a water-soluble analog of Camptothecin, in the form of multilamellar or unilamellar vesicles dispersed in an aqueous phase with a drug to lipid ratio (molar) of at least 1/100 and wherein the lipid is selected from phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, disphosphatidylglycerol (cardiolipin), phosphatidic acid, phosphatidylinositol, sphingolipids, glycolipids, sulfatides, lysolipids and fatty acids, sterols, polymerizable lipids, or combinations thereof; and wherein the lipid bilayer must contain either at least one charged lipid component present in an amount of at least 5% (molar), or the salt form of a water soluble analog in an amount of at least 5% (molar); or mixtures thereof.

28. The pharmaceutical composition according to claim 27 wherein the charged lipid is negative and is selected from phosphatidyl glycerol, a charged salt of cholesterol, or phosphatidylserine or mixture thereof.

29. The pharmacuetical composition according to claim 27 or 28 wherein 50% of the water soluble analog is incorporated into the lipid bilayer .

30. The pharmaceutical composition according to any of claims 27 to 29 wherein the lipid bilayer must contain either at least one charged lipid component present in an amount of at least 5 % (molar).

31. The pharmaceutical composition according to claim 27 or 28 wherein the charged salt of cholesterol is cholesterol suflate or hemi-succinate.

32. The pharmaceutical composition according to any of claims 27 to 32 wherein the lipid contains C18:1 or C18:2 phosphatidylcholine and/or phosphatidylglycerol, and optionally comprises cholesterol.

33. The pharmaceutical composition, according to any of claims 27 to 32, wherein the drug is toptecan.

34. The pharmaceutical composition, according to any of claims 27 to 33, wherein the surface of the liposome is modified by a polymer.

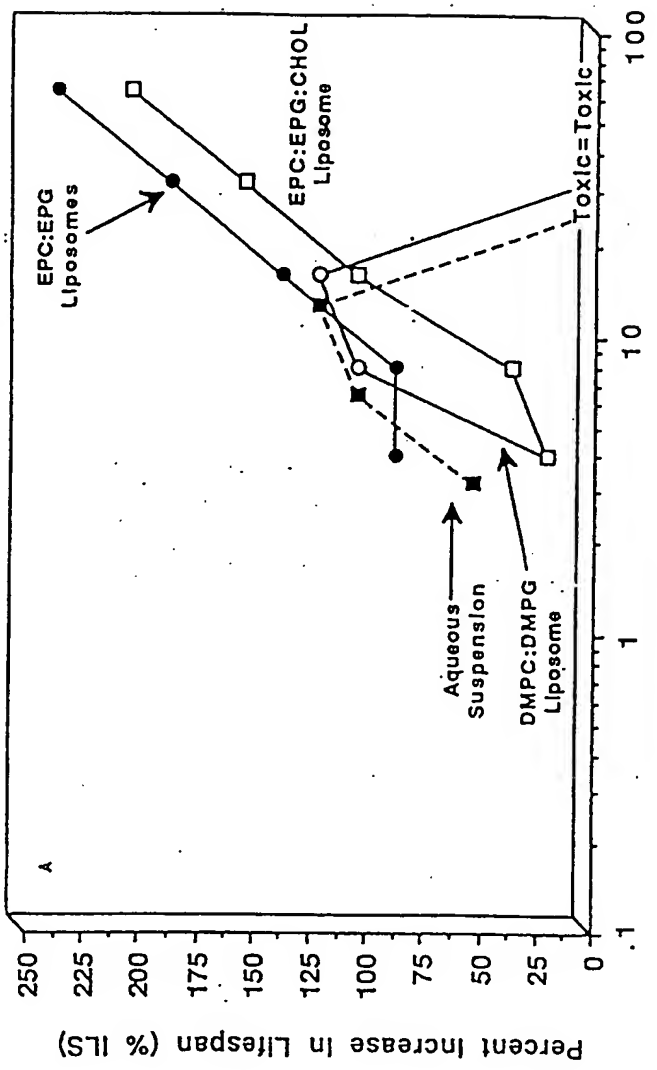
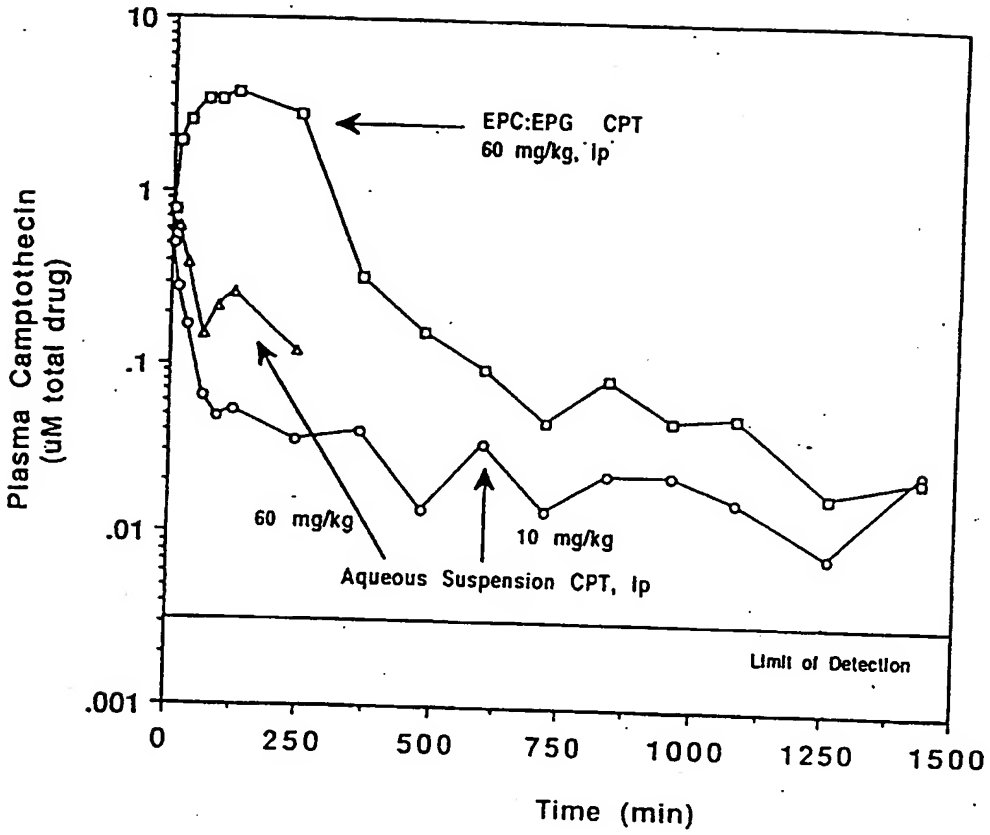

Percent Increase in Lifespan (% ILS)
250
225
200
175
150
125
100
75
50
25
0
.1
1
10
100
Toxic=Toxic
EPC:EPG
Liposomes
EPC:EPG:CHOL
Liposome
Aqueous
Suspension
DMPC:DMPG
Liposome
A

FIGURE 1

FIGURE 2


10
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.01
.001
Plasma Camptothecin
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60 mg/kg
10 mg/kg
Aqueous Suspension CPT, ip
Limit of Detection
0
250
500
750
1000
1250
1500
Time (min)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10898

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 9/127
US CL :424/450

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of American Chemical Society, Volume 114, 1992, (BURKE ET AL), "Liposomal Stabilization of Camptothecin's Lactone Ring", see pages 114-115.	1-15, 20, 25, 27-29
Y	Proceedings of National Academy of Sciences, USA, Volume 85, September 1988, (GABIZON ET AL) "Liposome formulations with prolonged circulation time in blood and enhanced uptake by tumors, see page 6949, column 2, Table 2 on page 6950.	7-9,15,20, 27,28, 32
Y	Biochimica et Biophysica Acta, Volume 1029, 1990, (BLUME ET AL), "Liposomes for the sustained drug release in vivo", see Abstract.	21-22,34

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10898

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Liposomes: From Biophysics to Therapeutics, (WEINSTEIN) "Liposomes in the Diagnosis and Treatment of Cancer", 1987, see pages 279, 280, 289, and 296.	7-9,15,20, 27,28,32
Y	US, A, 4,721,612 (JANOFF ET AL) 26 January 1988, see Abstract.	31